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TITLE: Methods for obtaining modified phenotypes in plant cells

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CLAIMS:

What is claimed is:

1. A method for reducing the phenotypic expression of a nucleic acid of interest, which is normally capable of being expressed in a plant cell, said method comprising the steps of a) providing to the nucleus of said plant cell unpolyadenylated RNA comprising a target specific nucleotide sequence produced by transcription of a chimeric DNA comprised within said plant cell, said chimeric DNA comprising a plant-expressible promoter operably linked to a target specific DNA region encoding said RNA, said target-specific DNA region comprising a nucleotide sequence of at least 10 consecutive nucleotides having at least about 70% sequence identity to about 100% sequence identity to said nucleic acid of interest and wherein said chimeric DNA farther comprises a DNA region involved in 3' end formation and polyadenylation, preceded by a self-splicing ribozymne encoding DNA region; and b) selecting said plant cell wherein the phenotypic expression of said nucleic acid of interest is reduced.

2. A method for reducing the phenotypic expression of a nucleic acid of interest, which is normally capable of being expressed in a plant cell, said method comprising the steps of a) introducing into the nuclear genome of said plant cell a chimeric DNA to generate a transgenic plant cell, said chimeric DNA comprising the following operably linked parts: i) a plant-expressible promoter region; ii) a target-specific DNA region comprising a nucleotide sequence of at least 10 consecutive nucleotides having at least about 70% sequence identity to about 100% sequence identity to said nucleic acid of interest; iii) a DNA region encoding a self-splicing ribozyme; and iv) a DNA region involved in 3' end formation and polyadenylation comprising a polyadenylation site

wherein said chimeric DNA when transcribed produces a first RNA molecule comprising a target specific nucleotide sequence and a self-splicing ribozyme, which when cleaved by autocatalysis produces a second RNA molecule comprising a target specific nucleotide sequence wherein the 3' end of the first RNA molecule comprising the polyadenylation site has been removed; and b) isolating a transgenic cell wherein said phenotypic expression of said nucleic acid of interest is reduced.

3. The method of claim 2, wherein said DNA region encoding a self-splicing ribozyme is located immediately upstream of said DNA region involved in 3' end formation and polyadenylation.

4. The method of claim 2, wherein said DNA region encoding a self-splicing

ribozyme comprises a cDNA copy of a self-splicing ribozyme from avocado sunblotch viroid, peach latent mosaic viroid, Chrysanthemum chlorotic mottle viroid, carnation stunt associated viroid, Newt satellite 2 transcript, Neurospora VS RNA, barley yellow dwarf virus satellite RNA, arabis mosaic virus satellite RNA, chicory yellow mottle virus satellite RNA S1, lucerne transient streak virus satellite RNA, tobacco ringspot virus satellite RNA, subterranean clover mottle virus satellite RNA, solanum nodiflorum mottle virus satellite RNA, velvet tobacco mottle virus satellite RNA, Cherry small circular viroid-like RNA or hepatitis delta virus RNA.

5. The method of claim 2, wherein said DNA region encoding a self-splicing ribozyme comprises a cDNA copy of a self-splicing ribozyme from barley yellow dwarf virus satellite RNA.

6. The method of claim 5, wherein said DNA region encoding a self-splicing ribozyme comprises the nucleotide sequence of SEQ ID No 1.

7. The method of claim 5, wherein said DNA region encoding a self-splicing ribozyme comprises the nucleotide sequence of SEQ ID No 2.

8. The method of claim 2, wherein said plant-expressible promoter is constitutive.

9. The method of claim 2, wherein said plant-expressible promoter is inducible.

10. The method of claim 2, wherein said plant-expressible promoter is tissue-specific.

11. The method of claim 2, wherein said nucleic acid of interest is a transgene.

12. The method of claim 2, wherein said nucleic acid of interest is an endogenous gene.

13. The method of claim 2, wherein said nucleic acid of interest is comprised within a virus or viral vector.

14. The method of claim 2, comprising the further step of regenerating a transgenic plant from said transgenic plant cell.

15. A chimeric DNA molecule for reducing the phenotypic expression of a nucleic acid of interest, which is normally capable of being expressed in a plant cell, said chimeric DNA molecule comprising a) a plant-expressible promoter region; b) a target-specific DNA region comprising a nucleotide sequence of at least 10 consecutive nucleotides having at least about 70% sequence identity to about 100% sequence identity to said nucleic acid of interest; c) a DNA region encoding a self-splicing ribozyme; and d) a DNA region involved in 3' end formation and polyadenylation comprising a polyadenylation site;

wherein said chimeric DNA when transcribed produces a first RNA molecule comprising a target specific nucleotide sequence and a self-splicing ribozyme, which when cleaved by autocatalysis produces a second RNA molecule comprising a target specific nucleotide sequence wherein the 3' end of the first RNA molecule comprising the polyadenylation site has been removed.

16. The chimeric DNA molecule of claim 15, wherein said DNA region encoding a self-splicing ribozyme is located immediately upstream of said DNA region involved in 3' end formation and polyadenylation.

17. The chimeric DNA molecule of claim 15, wherein said DNA region encoding a self-splicing ribozyme comprises a cDNA copy of a self-splicing ribozyme from avocado sunblotch viroid, peach latent mosaic viroid, Chrysanthemum chlorotic mottle viroid, carnation stunt associated viroid, Newt satellite 2 transcript, Neurospora VS RNA, barley yellow dwarf virus satellite RNA, arabis mosaic virus satellite RNA, chicory yellow mottle virus satellite RNA S1, lucerne transient streak virus satellite RNA, tobacco ringspot virus satellite RNA, subterranean

clover mottle virus satellite RNA, solanum nodiflorum mottle virus satellite RNA, velvet tobacco mottle virus satellite RNAvSCMoV or Cherry small circular viroid-like RNAcscRNA1.

18. The chimeric DNA molecule of claim 15, wherein said DNA region encoding a self-splicing ribozyme comprises a cDNA copy of a self-splicing ribozyme from barley yellow dwarf virus satellite RNA.

19. The chimeric DNA molecule of claim 18, wherein said DNA region encoding a self-splicing ribozyme comprises the nucleotide sequence of SEQ ID No 1.

20. The chimeric DNA molecule of claim 18, wherein said DNA region encoding a self-splicing ribozyme comprises the nucleotide sequence of SEQ ID No 2.

21. A plant cell comprising a nucleic acid of interest which is normally capable of being phenotypically expressed, further comprising the chimeric DNA of claim 15.

22. A plant comprising the plant cell of claim 21.

23. A method for identifying a phenotype associated with the expression of a nucleic acid of interest in a plant cell, said method comprising: a) selecting within said nucleic acid of interest a target sequence of at least 10 consecutive nucleotides; b) introducing a chimeric DNA into the nucleus of a suitable plant host cell comprising said nucleic acid of interest, said chimeric DNA comprising the following operably linked DNA fragments: i) a plant-expressible promoter region; ii) a target-specific DNA region comprising a nucleotide sequence of at least about 70% to about 100% sequence identity to said target sequence; followed by iii) a DNA region encoding a self-splicing ribozyme located immediately upstream of iv) a DNA region involved in 3' end formation and polyadenylation; and c) observing the phenotype by a suitable method.

24. A method for reducing the phenotypic expression of a nucleic acid of interest, which is normally capable of being expressed in cells of a plant, said method comprising the steps of a) introducing into the nuclear genome of cells of said plant a chimeric DNA to generate a transgenic plant cell, said chimeric DNA comprising the following operably linked parts: i) a plant-expressible promoter region; ii) a target-specific DNA region comprising a nucleotide sequence of at least 10 consecutive nucleotides having at least about 70% sequence identity to about 100% identity to said nucleic acid of interest; iii) a DNA region encoding a self-splicing ribozyme; and iv) a DNA region involved in 3' end formation and polyadenylation comprising a polyadenylation site

wherein said chimeric DNA when transcribed produces a first RNA molecule comprising a target specific nucleotide sequence and a self-splicing ribozyme, which when cleaved by autocatalysis produces a second RNA molecule comprising a target specific nucleotide sequence wherein the 3' end of the first RNA molecule comprising the polyadenylation site has been removed; b) regenerating a plant from said transgenic cell; and c) isolating said plant wherein said phenotypic expression of said nucleic acid of interest is reduced.

25. The method of claim 24, wherein said DNA region encoding a self-splicing ribozyme is located immediately upstream of said DNA region involved in 3' end formation and polyadenylation.

26. The method of claim 24 wherein said DNA region encoding a self-splicing ribozyme comprises a cDNA copy of a self-splicing ribozyme from avocado sunblotch viroid, peach latent mosaic viroid, Chrysanthemum chlorotic mottle viroid, carnation stunt associated viroid, Newt satellite 2 transcript, Neurospora VS RNA, barley yellow dwarf virus satellite RNA, arabis mosaic virus satellite RNA, chicory yellow mottle virus satellite RNA S1, lucerne transient streak virus satellite RNA, tobacco ringspot virus satellite RNA, subterranean clover mottle virus satellite RNA, solanum nodiflorum mottle virus satellite RNA, velvet tobacco mottle virus satellite RNA, Cherry small circular

viroid-like RNA, or hepatitis delta virus RNA.

27. The method of claim 24, wherein said DNA region encoding a self-splicing ribozyme comprises a cDNA copy of a self-splicing ribozyme from barley yellow dwarf virus satellite RNA.

28. The method according to claim 1, wherein said target-specific DNA region comprises a nucleotide sequence of at least about 20 consecutive nucleotides having about 100% sequence identity to said nucleic acid of interest.

29. The method according to claim 2, wherein said target-specific DNA region comprises a nucleotide sequence of at least about 20 consecutive nucleotides having about 100% sequence identity to said nucleic acid of interest.

30. A chimeric DNA molecule according to claim 15, wherein said target-specific DNA regioncomprises a nucleotide sequence of at least about 20 consecutive nucleotides having about 100% sequence identity to said nucleic acid of interest.

31. A method according to claim 24, wherein said target-specific DNA region comprises a nucleotide sequence of at least about 20 consecutive nucleotides having about 100% sequence identity to said nucleic acid of interest.